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STUDIES ON THE UTILIZATION OF DEBONED TROUT

(*Oncorhynchus mykiss*) FRAMES IN FISH SNACK

by

S. Muralidharan

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Nutrition and Food Sciences

Approved:

UTAH STATE UNIVERSITY
Logan, Utah

1999

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ABSTRACT

Studies on the Utilization of Deboned Trout

(*Oncorhynchus mykiss*) Frames in Fish Snack

by

S. Muralidharan, Master of Science

Utah State University, 1999

Major Professor: Dr. Conly L. Hansen
Department: Nutrition and Food Sciences

Snack food development studies were conducted to utilize trout (*Oncorhynchus mykiss*) frames, a by-product of the filleting operation, using extrusion and conventional technology. Twin screw extrusion studies were conducted to study the effect of fish mince, non-fat dry milk, process temperature, and moisture content on the physicochemical properties of the extruded snack food. Response surfaces were plotted to understand the effects of the independent variables on dependent variables such as bulk density, expansion ratio, shear strength, and water absorption index. Quadratic models expressed the relationship between the dependent and independent variables.

Based on the extrusion studies, conditions suitable for further development of a ready-to-eat snack food were obtained. Conventional technology was also studied in the development of a fish cracker called keropok. A well expanded, tasty snack food was obtained using the minced fish and tapioca starch. Physicochemical characteristics of the developed snack were determined. Taste panel ratings for texture and taste of the cracker indicated a good potential for acceptance of this product for production and sale by local fish processors. Further studies may be undertaken to develop a continuous process to prepare the crackers on a larger scale.

(106 Pages)

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S. Muralidharan

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LIST OF SYMBOLS

β_{ii}	Regression coefficients of the quadratic terms
β_{ij}	Regression coefficients of the interaction terms
β_o	Regression constant for the model
η	Regression equation
BKDEN	Bulk density
d.b.	Dry basis
DP	Die pressure
ER	Expansion ratio
F	Value of fish in the model
L/D	Length to diameter
M	Value of moisture in the model
N	Value of NFDM in the model
NFDM	Nonfat dry milk
PT	Product temperature
RSM	Response Surface Methodology
SME	specific mechanical energy
T	Value of Temperature in the model
WAI	Water Absorption Index

CHAPTER I

INTRODUCTION

Fish food is relatively low in calories and low in cholesterol (Gorga and Ronsivalli, 1988). Fish protein is well-balanced in essential amino acid composition and is easily digestible (Karmas and Lauber, 1987). Fish products have a low fat content compared to meat and poultry. Consumers' growing preference for a low fat, healthy food is opening a large demand for processed fish food. The annual per capita consumption of fish by Americans increased from 12.4 pounds in 1980 to about 15 pounds in 1995 (U.S. Bureau of the Census, 1997).

While processing fish into fillets and surimi, more than 50% of the fish by weight is lost. Lee (1986) reported that only 24% of the original fish mass is recovered as surimi. Martin and Collette (1990) found that the washed mince for the menhaden species amounted to only 20% of the original fish mass. The waste stream of these processes contains valuable fish protein, which could be removed in the form of minced fish using a mechanical deboner.

Fish protein can be processed into various foods, such as fish sticks, fish fingers, and snack products such as keropok (Murray *et al.*, 1980; Meinke *et al.*, 1983; Siaw *et al.*, 1985; Maga and Reddy, 1985; Karmas and Lauber, 1987; Reddy *et al.*, 1990). Various thermal processing methods such as deep fat frying, baking, and extrusion cooking are used to prepare these foods.

PROBLEM STATEMENT

Rainbow trout (*Oncorhynchus mykiss*) are raised commercially in Idaho and Utah. These two states account for over 80% of trout production in the U.S.A. Total sale value of trout amounted to $56.9 million in 1996 (Bureau of the Census, 1997). With the current processing technology, about one-half of the trout is discarded as waste in cleaning water, eviscerates, frames, heads, tails, and fins at small and medium-sized trout processing plants. Currently, trout frames (about 26% of the total waste) are buried under earth in alternate layers of frames and soil. This practice results in environmental problems and increases the market cost of the trout. Hence, it is desirable to have a process whereby some of the fish waste could be converted to a value added product. This would not only make the process competitive but would also reduce the environmental problems associated with trout processing.

One way of achieving this objective would be to utilize the protein from fish frames. The flesh in trout frames is about 85% by weight and can be removed by mechanical deboning.

This mechanically deboned flesh can be converted into an expanded fish snack using extrusion and conventional technologies.

OBJECTIVES

The overall objective of this study was to explore the possibility of utilizing deboned trout frames in the production of an expanded snack food. The specific objectives were as follows:

1. To conduct twin screw extrusion studies using wheat flour and deboned trout mince to obtain an expanded snack food.
2. To investigate the effects of formulation and extrusion parameters such as fish mince and process temperature on the physicochemical parameters of the extruded snack food.
3. To check whether or not conventional fish cracker production technology could be used for the development of an expanded snack, using the deboned trout muscle.

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CHAPTER II

LITERATURE REVIEW

This chapter presents the literature related to utilization of fish mince, especially for human consumption. Emphasis is given to conventional and extrusion technologies used for development of snack products, design methodologies involved, and characterization of the developed snack product.

Many unconventional fish species are caught during commercial fishing operations. In the past, they were used either as fertilizer or animal feed. A significant portion was also dumped into the sea for lack of other disposal options. Availability of mechanical deboners, which separates the nutritious fish muscle from the bones, led to the possibility of using the minced fish obtained from these fishes to produce value-added products.

Venugopal and Shahidi (1995) explored the various possibilities available for product development using fish mince from the unconventional fish species. Some of the possible uses were surimi and surimi-based products, sausages, fermented products, protein concentrates, hydrolysates, and extruded products. However, the authors cautioned that similar results cannot be expected from the process for all varieties and species of fish. Venugopal (1987) alerted product developers that incorporation of whole fish meat could give rise to products having objectionable fishy flavor, and suggested using fish mince in conjunction with starchy materials for developing snack products. Almost all of the research conducted with the objective of preparing fish snacks and imitation products have used a carbohydrate source in addition to the minced fish.

CONVENTIONAL PROCESSING OF FISH MINCE

Fish crackers, cutlets, creamy fish bites, fish balls, fish fingers, and sausages are some of the snack foods that have been prepared using the deboned minced fish of various species (Venugopal and Shahidi, 1995).

Fish crackers, called keropok are a very popular snack food in Malaysia and other South East Asian countries. The crackers are produced by mixing fish mince and flour to form a dough, which is shaped, cooked with water to gelatinize the starch, and sliced. These slices are then dried to get a low-moisture intermediate product, with good shelf life. A low density expanded snack for consumption is obtained by immersion into hot oil (Siaw *et al.*, 1985). Fish, prawns, and other ingredients are added to give the desired flavor to the snack food.

Okraku-Offei (1974) used the conventional technology as explained above with several species of fish (grouper, gray snapper, and red snapper) caught in Ghana. Shelf-life studies indicated that low-moisture intermediate products were good for a year, and ready-to-eat fried products could be sealed and stored at refrigerator temperature for a month. Taste panel studies conducted with six trained panelists resulted in high scores for crispness and flavor of the snack.

Siegel *et al.* (1976) reported the use of fish protein concentrate in the development of a snack food similar to rice noodles. The product was steamed, dried, and deep fat fried before use. Among Thai school children this snack had a higher sensory score than a similar commercially available snack had.

Siaw *et al.* (1985) developed a mechanical process, based on sausage technology, to produce keropok of better quality and consistency than the ones obtained using the traditional method. It involved stuffing the mix into casings and steaming to gelatinize the product. Casings were cooled overnight, sliced, and dried to get an intermediate product that could be fried to get the desired snack. This process was used later by many other researchers for their studies of keropok. Taste panel studies conducted by Siaw *et al.* found no significant difference between the product made using this new mechanical method and the traditional nonmechanical method.

Yu and Tan (1990) prepared keropok using fish protein hydrolysate obtained from minced *Oreochromis mossambicus. Oreochromis mossambicus is* a readily available fresh water fish of South East Asia. An enzyme, alkalase, was used to hydrolyze the fish. Crackers were prepared using the method developed by Siaw *et al.* (1985). Ten percent hydrolysate was found to give maximum linear expansion (ratio of increase in length to initial length) of the crackers. There were no sensory differences between the cracker containing minced fish and crackers made using the fish protein hydrolysate.

Yu (1991a, b) studied the effect of fish:flour ratio and the effect of different types of flour on the preparation of crackers, using locally available fish species (*clupea leiogaster* and jew fish). He observed a marked decrease in product expansion when the protein content was higher than 30% and suggested a maximum of 2:1 fish:flour ratio. He also reported a high acceptability score for crackers prepared using tapioca flour. Haryadi

(1994) studied six different starches and tested their suitability for use in the manufacture of fish crackers and found that different starches gave different extents of expansion. The expansion did not correlate with the amylopectin content. However higher amylopectin content of the starch gave more acceptable falvor to cracker. Of all the starches used, tapioca starch gave the maximum expansion and highest sensory acceptability. When a good basic cracker has been developed, addition of various flavor products such as bacon, cheese, fish, or spices can be tried to obtain a marketable snack cracker.

Yu and Low (1992) studied the possibility of using pregelatinized tapioca starch in the production of keropok, with the objective of reducing the steaming process time. Only keropok made from a slurry with a water:starch ratio of 70:30 and pregelatinized at 133.5°C was found acceptable.

Yu (1992) explored the possibility of replacing the widely used *Rastrelliger kanagurta* fish with a fresh water fish *Oreochromis mossambicus* in the keropok snack. A replacement of not more than 60% was found to be acceptable by the taste panelists. Thus, the type of species used is an important factor in the fish crackers. He also reported that a slice thickness of 3.0 to 3.5 mm was most acceptable to taste panelists (Yu, 1993).

Meinke *et al.* (1983) reported the fortification of fish mince with soy protein in the development of fish sticks. Regular stick-shaped pieces of frozen mince were cut using a band saw, covered with batter and breading material, and prefried and frozen. The sticks were deep fat fried prior to consumption by taste panel members. A ratio of 3.3:1 fish mince:soy protein resulted in fish sticks with good sensory attributes.

Bartholomew *et al.* (1983) used a manual deboning process to separate the flesh from rainbow trout (*Oncorhynchus mykiss*) to produce a canned flake-style product. However, the process could not be commercialized because of the problems faced in mechanizing the deboning of the mince. Mechanically deboned mince did not give an acceptable flake.

Buck and Fafard (1985) reported the use of minced red hake in the preparation of fish sausage. Minced fish was mixed with spices and liquid smoke to obtain a fish sausage. Taste panel studies that compared fish sausage with beef and pork sausages resulted in the lowest acceptability score for the fish sausage.

Product development activities conducted at Cornell University using minced fish resulted in meat-like products (Regenstein, 1986). A mixture of fish mince and starch was canned, retorted, sliced, and deep fat fried to get a snack product. However, process details and customer acceptability were not reported.

From the above studies it can be concluded that a good potential exists for the development of snack food using minced trout and starchy materials. Tapioca starch has been a basic ingredient in most of the snack food studies that have targeted the utilization of low fat fish in the development of the snack foods.

EXTRUSION PROCESSING OF FISH MINCE

Twin screw extrusion technology for food processing started in the 70's and expanded to many applications in the 80's (Harper, 1989). Extrusion processing involves forcing moistened proteinaceous and/or starchy food materials to flow under a variety of process conditions in a food extruder and finally through a specially designed die opening at a predetermined rate (Harper, 1981). Gelatinization of starch, denaturation of protein, protein texturization, and composite structuring of starch and protein are the major changes that occur during food extrusion (Clayton and Miscourides, 1992). The degree to which these changes take place during the extrusion process determines the texture and structure of the extruded product. The use of extruders for the processing of fish muscle is relatively new. Choudhury and Gogoi (1995) reviewed the use of extruders in utilizing fish mince and concluded that a systematic study of the physicochemical changes occurring in food material during extrusion and the interrelationship between process variables is lacking.

Extrusion studies using fish mince

The earliest study using Atlantic cod mince and soy protein to understand the extrusion process was conducted by Murray *et al.* (1980). Preprocessing of the mince involved mixing of the mince with salt water and centrifuging. Centrifuged mince was desalted by washing with water, and the mince was freeze dried to reduce the moisture content. They studied the effects of compositional and processing variables on the extruded products, using a control composite response surface design. Protein water ratio (P/W), vegetable fish protein ratio (V/F), and processing temperature had a significant effect on the texture and other characteristics of the extruded product. Sensory analysis of the product attributed fish odor of snack as a reason for the low taste panel scores.

Bhattacharya *et al.* (1990, 1993) studied the effect of extrusion process variables on the texture and micro structure of the extruded products, using a single screw extruder. Bombay Duck (*Harpodan nehereus*), a low cost fish available locally, was deboned, and the mince was mixed with wheat flour for the extrusion study. Pre-processing of the mince involved partial drying at 60-65°C in a tray drier to facilitate the extrusion process. The process variables examined were extruder barrel length to diameter (L/D) ratio, feed ratio, extrusion temperature, and screw speed. Extruded rods were cooked, and the textural properties of the cooked rod were compared with cooked fish. The extruded product had a greater hardness as compared to the cooked fish. Hardness, springiness, and cohesiveness were significantly affected by L/D ratio and feed ratio. The authors concluded that the effects of screw speed and temperature on product texture were complex in nature. Scanning electron microscopy studies were done to study the micro structure of the extrudates. A lower L/D ratio was suggested for the development of an expanded snack. In their previous study (Bhattacharya *et al.*, 1988) these same researchers had noted a slight increase in protein digestibility values of fish and flour blends as an effect of the extrusion process. Of the process variables, only temperature and feed ratio affected the protein digestibility of the extrudates.

Clayton and Miscourides (1992) generated preliminary process design information for the production of an expanded snack food and a meat substitute using Atlantic cod (*Gadus morhua*) mince and rice flour. Preprocessing of the mince involved washing with water, deboning, washing with ethyl alcohol, pressing to remove the moisture, and then drying in order to make a fish flour. A single screw Haake Rheodrive extruder was used in the study. An orthogonal rotatable central composite design was used in the design of experiments. The researchers generated the response surfaces, which enabled them to understand the effect of independent variables (moisture content, protein content, temperature, and screw speed) on the extrudate's characteristics. They suggested a processing temperature of 180°C, moisture content of 25% (d.b), and protein content 18% (d.b.) for the development of an expanded snack food.

In 1994, Choudhury applied the extrusion technology to process fish muscle and reported the effects of moisture content, feed ratio, and feed rate on the extrudate characteristics. A processing temperature of 160°C was used in his study. A central composite rotatable design was used and quadratic relationships were determined between the independent variables and the dependent variables such as specific mechanical energy and die pressure. He concluded that feed rate had little influence on product characteristics.

Gogoi *et al.* (1996a, b) investigated the effect of the location and spacing of the reverse screw elements on specific mechanical energy and product attributes of the extrudates obtained from minced pink salmon fish and rice flour. A co-rotating Clextral twin screw extruder was used in the study. Fish were dried at 60°C for 8 hr in a tray drier prior to extrusion. Reverse screw elements increased specific mechanical energy and expansion ratio, but decreased bulk density of the extrudates.

Gautam *et al.* (1997) investigated the effect of type of mixing element and feed composition effects on specific mechanical energy and product attributes. Three types of mixing elements, kneading, reverse screw, and combination of kneading and reverse screw, were studied. A high expansion ratio was obtained using the kneading element. However, reverse screw and the combination of kneading and reverse elements reduced the expansion ratio. Increased protein content reduced the expansion ratio. Predrying of the minced pink salmon was necessary prior to extrusion and a Clextral twin screw extruder was used in the study.

Choudhury *et al.* (1998) studied the effect of kneading elements on specific mechanical energy and extrudate characteristics. A twin screw Clextral extruder was used to extrude the blends of minced pink salmon and rice flour. They reported an increase in specific mechanical energy, expansion ratio, and water solubility index due to incorporation of the kneading elements. However, a reverse trend was observed in those parameters because of an increase in fish content.

From the above reviews the following conclusions can be drawn. Twin screw extruders are increasingly used for extrusion processes because of their versatility. Systematic study to learn the effect of various independent process parameters is fairly recent. Pre-drying of the minced fish seems to be a requirement to accompish the extrusion process. L/D ratio, process temperature, moisture content, screw configuration, and screw speed are some of the parameters that need to be studied to understand the extrusion process. Central composite rotatable designs have been used in the experimental design.

snack. A significant difference in taste was observed in the sensory panel tests when the fish content was higher than 35%.

Venugopal (1987) reported boiling of minced croaker fish (*Johinius dossumuri*) in orthophosphoric acid to deodorize it. The deodorized meat was mixed with wheat flour, salt, oil, and water and extruded using a single screw extruder to get a snack food. However, details of the process were insufficient for the duplication of the process. Also, no taste panel studies were conducted.

Quaglia *et al.* (1989) conducted a preliminary study using sardine mince, rice, wheat, and corn to produce pasta and snacks using a single screw extruder. Extruded snack had good expansion properties when deep fat fried in peanut oil. They suggested further organoleptic evaluation of the product.

Julianty *et al.* (1994) explored the use of egg white powder (EWP) as a protein source when extruding fish crackers using a single screw extruder. Extrudates were microwaved before consumption. Up to 3% EWP could be added without any significant reduction in product exapansion.

Choudhury *et al.* (1995) discussed the possibilities and prospects of twin screw extruders in the development products were made using the extruded meat sol. Toughness of the jelly was evaluated by a sensory panel. They reported a higher screw speed (400 rpm) and moderate feed rate of fish (20 kg/hr) to obtain extrudates gave the gel a similar texture as that obtained using ground fish paste.

Chen (1993) developed an extrusion process for converting small-sized, underutilized fish particles into fillet analogues. Carboxy Methyl Cellulose (CMC) and Xanthan were used as binding agents. A cooled die extension was used to avoid puffing of the extrudate. The extrudate was analyzed for shear stress, cooking loss, and oil uptake. There was no significant difference between the physical properties of the fish fillet analogues and natural fish fillets.

RESPONSE SURFACE METHOD

Response surface methodology describes the relationship between the product quality characteristics and the process parameters. It is based on regression analysis of quantitative data from appropriate experimental designs to solve multivariate equations (Olkku *et al.*, 1983). These equations are used to map the response surfaces. Response surfaces aid in understanding the effect of process variables on product characteristics.

A second-order model adequately describes changes in an extrusion process (Eerikainen and Linko, 1989). A second-order model is of the form

$$\eta = \beta_o + \sum \beta_i x_i + \sum \beta_{ii} x_i x_i + \sum \sum \beta_{ij} x_i x_j$$

and has been used in extrusion research involving fish mince (Murray *et al.*, 1980; Bhattacharya *et al.*, 1988; Clayton and Miscourides, 1992; Choudhury, 1994).

PHYSICOCHEMICAL CHARACTERISTICS

Some of the physicochemical characteristics used to characterize snack foods include:

Linear expansion

Expansion ratio

Bulk density

Water absorption index (WAI)

Shear strength

Die pressure

Specific mechanical energy (SME)

Linear expansion

Linear expansion describes degree of expansion of the product upon frying in oil. It is a measure of the crispiness, the most important sensory attribute of fish crackers (Yu and Tan, 1990). This property has been used as a measure of the quality of keropok by almost all researchers from Malaysia and other South East Asian countries.

Expansion ratio

Expansion ratio (ER) governs the texture and mouth feel of puffed extruded snacks (Owusu-Ansah and Van de voort, 1984). Expansion of the extruded product can take place both radially and longitudinally. Different methods have been used by researchers to calculate ER. Mercier and Feillet (1975), Gogoi *et al.* (1996a), and Gautam *et al.* (1997) used the ratio of area of the extrudate to the area of the die as ER. Faubian and Hoseney (1982) expressed ER as the ratio of the diameter of the extrudate to the diameter of the die opening of the extruder. Clayton and Miscourides (1992) considered the diameter of the extrudate itself as a response.

Bulk density

Bulk density is an indicator of the lightness of the product. Different methods of measurement have been reported because of the irregular shape of the snack products. Lue *et al.* (1991) and Goga (1997) used fine sand as a filler material. Weighed extrudates were placed in a beaker of known weight and volume. Fine sand whose bulk density is known is added to the spaces between the extrudates and compacted well by tapping. From the volume of displacement and weight of the extrudates, bulk density of the extrudate was determined. Gautam *et al.* (1997) reported a different method for finding the volume of the extrudates. Average diameter of the extrudate was determined by measurement and volume was calculated as the product of cross sectional area and length of extrudate. Singh (1994) determined bulk density by grinding the product to a specific size and then compacting the product into a known volume.

Water absorption index (WAI)

Water absorption index (WAI) is a measure of the swelling power of starch. Wang *et al.* (1993) reported that ungelatinized starch will absorb only a small amount of water. It is generally agreed that WAI is a good indicator of the gelatinization of starch during the extrusion process.

WAI is determined by the method developed by Anderson *et al.* (1969) in most of the studies. This method involves suspending 2.5 g of the ground (<60 mesh) product in 30 ml of water at 30°C in a 50-ml tared centrifuge tube. The product is kept in a water bath for 30 min and stirred intermittently. After the 30-min period, it is centrifuged at 3000 g for 10 min and the supernatant poured off. The weight of gel per gram of dry sample yields WAI.

Shear strength

Shear strength (SS) indicates the amount of force needed to shear the extruded snack and is an indicator of the texture of the product. A lower value indicates a lighter and more expanded product. A very popular method of measuring the shear strength is using a Warner-Bratzler apparatus attached to a texture analyzer. Maurice *et al.* (1976) reported a very strong correlation between the instrumental and sensory measures of shear.

Die pressure

Die pressure (DP) indicates how the product is behaving in the barrel (Goga, 1997). The high temperature and pressure conditions cause the water in the extruder to exist in a superheated form (Williams *et al.*, 1977). The pressure differential between the mix before the die opening and atmospheric pressure causes the product to expand soon after exiting the die. Cooling causes texturization and contraction of the product.

Specific mechanical energy

Specific mechanical energy (SME) relates to the specific work input from the motor to the material being extruded and is calculated using the formula

$$SME = \frac{N * T * K_w}{Q} \quad (KW\ h/kg)$$

where

N = per cent screw speed

T = per cent torque

K_w = motor power, in KW

Q = Feed rate (Kg/h) (Frame, 1994).

This value depends on the product recipe. SME is a function of temperature, moisture, feed rate, and shear rate (Goga, 1997).

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CHAPTER III

EXTRUSION PROCESSING OF FISH FROM DEBONED TROUT FRAMES[1]

ABSTRACT

We investigated formulation and processing temperature to determine their effect on physicochemical characteristics of an extruded snack made with deboned trout. Formulation parameters included feed moisture content (20% - 30%), nonfat dry milk (NFDM) (0% - 5%), and dried trout fish mince (0% - 2%). Three temperatures (125°C, 150°C, 175°C) were tried. The physicochemical parameters investigated were bulk density (BKDEN), expansion ratio (ER), water absorption index (WAI), shear strengh (SS), specific mechanical energy (SME), die pressure (DP), and product temperature (PT). Response surface methodology was used to analyze the data and generate quadratic models that describe the relationships between the independent formulation and processing variables to the dependent physicochemical characteristics of the extrudates. Moisture content had a linear effect

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[1] Coauthored by S. Muralidharan, C. Hansen, J. Irudayaraj and C. Carpenter.

($p < 0.01$) on all the measured physicochemical characteristics of the extrudates. Extrusion temperature affected BKDEN, WAI, SS, SME, DP, and PT ($p < 0.01$). Fish content affected ($p < 0.001$) dependent variables SS, BKDEN, ER and SME. NFDM affected ($p < 0.01$) SS, WAI, and SME. The experiments indicated that, among the extrudates containing fish, product obtained under conditions of 175°C, 20% moisture, 0% NFDM, and 2% dried fish gave a uniformly expanded product of low bulk density.

INTRODUCTION

The fish processing industry is a major supplier of nutritious food, but the processing operation generates a large amount of wastes. The waste stream from filleting, canning, and surimi operations includes fish trimmings, belly flaps, heads, frames, fins, skins, and viscera. However, a significant amount of these fishery by-products is not utilized due to the lack of technology to transform them into acceptable products. Currently, only a small fraction of the waste is converted to fish meal; the remainder is milled and dumped into nearby marine waters (Choudhury and Gogoi, 1995). The frames of fish can be mechanically deboned to give a fish meat mince. However, minced fish lacks texture, and hence has poor marketability. Venugopal *et al.* (1992) reviewed various options for utilizing the fish mince as flour or protein hydrolysate, each of which is incorporated into a variety of fermented foods, fabricated foods, and extruded foods.

Rainbow trout (*Oncorhynchus mykiss*) is a fresh water fish raised commercially in the states of Utah and Idaho. These two states account for over 80% of the total trout production in the U.S.A. During 1996, the annual production of trout amounted to 53.6 million pounds valued at $57 million (U.S. Bureau of the Census, 1997). The waste stream from small and medium-sized trout processing plants is disposed of by burying in soil, adding to environmental pollution. It is necessary to find a way to utilize the fish mince obtainable from these trout frames.

Extrusion processing is widely used in the food industry in the manufacture of products such as pasta, noodles, and ready-to-eat (RTE) cereals. An extruder is basically a screw pump of tightly fitting flighted screws rotating in a stationary barrel. This unit functions as a scraped surface heat exchanger that shears, heats, texturizes, shapes, and puffs the raw materials (Choudhury and Gogoi, 1995).

There are many studies reporting extrusion of fish mince. Early studies concentrated on producing a specific product rather than understanding the physicochemical effects of the extrusion process. Fish mince has been extruded into intermediate moisture foods, fillet analogues, and expanded snack foods (Yu *et al.,* 1981; Maga and Reddy, 1985; Karmas, 1985; Karmas and Lauber, 1987; Kitabatake *et al.,* 1988; Quaglia *et al.,* 1989; Chen, 1993).

More recently, systematic research has provided basic information that relates process variables to physicochemical changes that affect product quality (Murray *et al.,* 1980; Bhattacharya *et al.,* 1990, 1993; Clayton and Miscourides, 1992; Choudhury, 1994, Choudhury *et al.,* 1995; Gogoi *et al.,* 1996; Gautam *et al.,* 1997). Some of the more important processing variables include moisture content, fish content, extrusion process temperature, extruder screw speed, and L/D ratio. Some important physicochemical characteristics measured as related to quality include bulk density, expansion ratio, shear strength, water absorption index, specific mechanical energy, and die pressure.

However, very little information was available for the use of wheat flour and trout mince. We also believed that the addition of NFDM would impart desirable textural attributes to the extruded snack food. Thus, this study investigated the effects of formulation and extrusion process temperature on an extruded, ready-to-eat snack food containing up to 2% trout mince on dry basis. The formulation variables were the fish content, moisture content and NFDM content.

MATERIALS AND METHODS

Materials

Trout frames, a by-product of the filleting operation, were deboned using a mechanical deboner (model RSTC-02 BX-V05, Beehive Machinery Inc., Sandy, UT). Frames were from Road Creek Ranch (Loa, UT) and Paradise Trout Farms (Paradise, UT). Deboned fish mince (2 kg portions) were collected in polyethylene bags and stored at -28.8°C (-20°F) until further use. Frozen mince was thawed for about 24 hr at 4°C, before use.

All purpose wheat flour (Pillsbury Inc., Minneapolis, MN) was obtained from a local market. Grade A pasteurised non fat dry milk (NFDM)(Farmers Cooperative Creamery, McMinnville, OR) was obtained from the G.H. Richardson Dairy Products Laboratory, belonging to the Nutrition and Food Sciences Department, Utah State

University. Proximate analysis of the deboned trout mince was determined using standard AOAC procedures (1990). Ether extraction method as described by procedure 960.39B was used for the determination of fat content. Vacuum oven method as described by procedure 950.46A was used to determine moisture content. Oxidation method using a muffle furnace was used to determine the ash content (procedure 920.153). Because fish do not contain carbohydrates, the protein content was determined as the difference between 100 - (percentage composition moisture, fat and ash).

Experimental design

Response surface methodology (ECHIP version 6.0 for windows, ECHIP Inc., Hockessin, DE) was used to design the experiment and analyze the data. A central composite design was used due to its efficiency and symmetricity. This design consisted of 16 factorial points, 8 extra points to form the central composite design, and 6 replications for the estimation of the process variability. The software generated the quadratic model based on the regression analysis of multivariate equations. ECHIP generated equations of the form

$$\eta = f(x_1 x_2 \ldots x_k) \qquad (1)$$

in terms of coded variable x, where

$x_i = 2(\xi_i - \zeta i)/d_i$

where

ξ_i = actual value in original units

ζi = mean of the high and low levels of ξ_i.

d_i = difference between low and high levels of ξ_i.

The value of the coded variable levels varies between ±1.

The dependent variables for this study were product temperature (PT), die pressure (DP), specific mechanical energy(SME), bulk density (BKDEN), water absorption index (WAI), expansion ratio (ER), and shear strength (SS).

Based on our subjective evaluation of product expansion, air cell distribution, and texture of extrudate, the ranges of values for independent variables were established in preliminary experiments as follows:

Die temp (T): 125 - 175°C.

NFDM content (N): 0% - 5%.

Dried fish content (F): 0% - 2%.

Moisture content (M) :20% - 30%.

The extrudate with no NFDM and dried fish content acted as a control. Addition of more than 2% dried fish to the ingredients gave an extrudate with unacceptable fishy flavor and hard texture. Based on the above variables, the amount of wheat flour varied from a minimum of 63% to the maximum of 80%. Other extrusion conditions that were held the same for all the extrusion runs were as follows:

Feed rate	:	4 kg/hr
Extruder screw rpm	:	500
Die opening (dia.)	:	1.9 mm

The trials to be conducted as generated by the ECHIP software are shown in Table 3.1. In order to maintain the randomness of the experiment, the trials were conducted in the same order as generated by the software.

Extrusion

An APV Baker MP19 co-rotating, twin screw extruder (Model # MP19, APV Baker Inc., Grand Rapids, MI) suitable for product development was used for the extrusion studies. The L/D ratio of the extruder was 25. It had the capability to mix and match the various components of the screw elements such as paddles, feed screw, and lead screw on the splined shaft. A high shear configuration was used for this study. The details of the screw configuration are shown in Table 3.2.

The predetermined amounts of dry ingredients (wheat flour, NFDM and dried fish mince) were mixed thoroughly

Table 3.1--Levels of independent variables as generated by ECHIP® software for the central composite design

Run No.	% Moist.	% Fish	% NFDM	Barr. Temp.
1	20.0	0.0	0.0	175.0
2	25.0	1.0	2.5	150.0
3	20.0	2.0	5.0	125.0
4	20.0	0.0	5.0	175.0
5	20.0	0.0	0.0	125.0
6	20.0	2.0	0.0	175.0
7	20.0	2.0	5.0	175.0
8	20.0	1.0	2.5	150.0
9	25.0	1.0	2.5	150.0
10	30.0	0.0	5.0	125.0
11	25.0	1.0	2.5	150.0
12	30.0	0.0	0.0	175.0
13	25.0	1.0	0.0	150.0
14	30.0	2.0	5.0	175.0
15	30.0	0.0	5.0	175.0
16	20.0	0.0	5.0	125.0
17	30.0	0.0	0.0	125.0
18	25.0	1.0	5.0	150.0
19	25.0	1.0	2.5	150.0
20	25.0	2.0	2.5	150.0
21	20.0	1.0	2.5	150.0
22	30.0	1.0	2.5	150.0
23	25.0	1.0	2.5	125.0
24	30.0	2.0	5.0	125.0
25	25.0	0.0	2.5	150.0
26	25.0	1.0	2.5	175.0
27	20.0	2.0	0.0	125.0
28	30.0	2.0	0.0	125.0
29	25.0	1.0	2.5	150.0
30	30.0	2.0	0.0	175.0

Table 3.2--Configuration of the twin screws used in the study

Screw Elements Type	Length in terms of diameter
5 D twin lead Screw, 1 D pitch	5.00
7 x 30 forwarding 1/4 D paddles	1.75
3 D twin lead screw	3.00
4 x 30 forwarding 1/4 D paddles	1.00
2 x 90 1/4 D paddles	0.50
2 D twin lead screw, 1 D pitch	2.00
2 x 30 forwarding 1/4 D paddles	0.50
2 D twin lead screw, 1 D pitch	2.00
5 x 60 forwarding 1/4 pitch	1.25
4 D twin lead screw, 1 D pitch	4.00
4 x 60 forwarding 1/4 D paddles	1.00
4 x 30 reverse 1/4 D paddles	1.00
2 D single lead screw	2.00

using a Waring blender. Then the mix was introduced into the feed zone, using a volumetric feeder (K-Tron Institute, Pitman, NJ). Water was introduced into the feed zone using a positive displacement pump (Bran+Luebbe Inc., Buffalo Grove, IL). Both the dry ingredient feeder and the positive displacement pump were calibrated before every run in order to determine the feed rate settings.

Barrel temperature was at room temperature near the feeding zone and maintained at the required temperature at the die point. The heating area consisted of four zones, and the temperature maintained at each zone during different temperatures of processing is shown in Table 3.3.

Extrusion trials were begun once the extruder barrel reached the required temperature. Water was introduced to the barrel to transport the ingredients to the die end. Dry ingredients were fed at a slow rate to start with. Feed rate of dry ingredients was increased, and the moisture level was decreased to attain the desired moisture and feed rate.

Samples were collected once a steady state was achieved, as indicated by constant torque, die pressure, and extrudate temperature. Long strands of the extrudates were collected on a tray and allowed to cool and harden

Table 3.3--Barrel temperature profile maintained along the extruder

Die	Zone			
temperature °C	Z4	Z3	Z2	Z1
125	100	75	50	25
150	120	90	60	25
175	140	105	70	25

Z1: Temp. near the feed zone.
Z4: Temp. near the die zone.

overnight. Cooled strands were broken into smaller lengths and were stored in Ziploc® (Dow Brands L.P., Indianapolis, IN) gallon-size freezer bags and held at 4°C until further analysis. Shut-down procedures followed the manufacturer's recommendations, after sample collection. The screws and barrel were cleaned thoroughly and lubricated with food-grade lubricant to avoid microbial contamination.

Physicochemical properties

Collected samples were analyzed for the following: bulk density, expansion ratio, shear strength, water absorption index, product temperature, die pressure, and specific mechanical energy.

Bulk density

Bulk density of the extrudates was determined using the method reported by Lue *et al.*(1991). White sand, whose density had been calculated beforehand, was used to determine the volume of the extrudate. Approximately 10 g of the extrudates, whose length and weight were measured beforehand, was placed in a beaker of known weight and volume. Sand was poured into the remaining space in three to four increments. The beaker was tapped after each increment to ensure the packing of sand in the remaining space and leveled off using a straight edge. From the volume of displacement and weight of the extrudates bulk density of the extrudate was determined. Average bulk density calculated from five determinations for each run was used in the analysis of the response surface.

Expansion ratio

Expansion ratio was determined by dividing the average diameter of the strand by the diameter of the die orifice, as reported by Faubian and Hoseney (1982). Diameter of the extrudate was determined using the formula

$$D = \sqrt{\frac{4 * V}{3.142 * L}} \qquad \ldots\ldots\ldots\ldots\ldots\ldots(2)$$

where

D = Average diameter of the extrudates (m)

V = total volume of the extrudates (m^3)

L = total length of the extrudates (m)

Shear strength

A texture analyzer (QTS-25, Stevens Advanced Weighing Systems Inc., Essex, UK) was used for the determination of shear strength. The procedure outlined by Maurice *et al.* (1976) was followed for the determination of shear strength. A Warner-Bratzler shear attachment was used for the shearing of extrudates. A shearing rate of 500 mm/min was used. Shear stress reported was the average of 15 individual strands for each extrusion experiment.

Water absorption index

Water absorption index (WAI), which is a measure of the degree of gelatinization of the product, was determined using the procedure reported by Anderson *et al.* (1969). A 2.5-g sample of the product was ground (<60 mesh) and suspended in 30 ml of water at 30°C in a 50-ml tared centrifuge tube. The product was kept in a water bath for 30 min and stirred intermittently. After the 30-min period, it was centrifuged at 3000 g for 10 min and the supernatant poured off. The weight of gel per gram of dry sample yielded the WAI.

Specific mechanical energy

Specific mechanical energy (SME) was calculated using the formula reported by Frame (1994).

$$SME = \frac{N * T * Kw}{Q} \quad (KW\ h/kg) \ \ldots\ldots\ldots\ldots\ldots (3)$$

where

N = rpm run/rpm rated for the screw.

T = per cent torque

K_w = rated motor power, in KW

Q = Feed rate (Kg/h)

SME was calculated based on the torque reading recorded at steady-state conditions of each extrusion run. The above formula reduced to

$$SME = T/200 \ (KW\ h/kg)$$

Product Temperature and Die pressure

Product temperature and die pressure readings were recorded at the time of extrusion, when the steady-state condition was reached for each run.

RESULTS AND DISCUSSION

The proximate analysis of the deboned trout mince is replicates.

During the extrusion trials using wet mince, uneven flow of feed material occurred leading to unstable extrusion conditions. So fish mince was dried to about 5% moisture (w.b) in a dryer ((model#1640 Shelldon Manufacturing Inc., Cornelius, OR) at 60°C (140°F) for 12-14 hours. The dried fish mince was used in all the experiments (Bhattacharya *et al.*, 1993; Clayton and Miscourides, 1992; Choudhury, 1994; Gautam *et al.*, 1997).

Table A.1 includes the experimental data obtained for various dependent variables. Table A.2 includes a summary of results showing the statistical significance of each term in the generated quadratic models to explain the effect of the independent variables. Appendix B (Figures B1 - B7) shows the effects graph for each dependent variable. The range associated with each independent variable is marked on these figures. Overlapping ranges indicates that one variable's responses were not statistically significant ($p > 0.05$) from those of another.

Bulk density

The following model explained 87.6% of the

Table 3.4--Proximate composition of deboned trout (*Oncorynchus mykiss*) mince

Composition	Percentage (Mean±SD)
Moisture	72.4±0.7
Fat	10±2
Protein	17±1
Ash	0.73±0.01

variability in BKDEN due to the variation of independent variables. Table 3.5 lists the mean squares of all the terms.

BKDEN = 268.17 + 15.19M + 148.2F - 3.92T - 0.523M*T - 2.86 F*T ....(4)

Bulk density is a measure of the lightness of the product, and for an expanded snack food a lower bulk density is desired. Measured bulk density varied from 81.51 Kg/m^3 (20% moisture, 0% fish, 0% NFDM, and 175°C) to 1008.33 kg/m^3 (30% moisture, 2% fish, 5% NFDM, and 125°C). The linear effect of temperature and fish content was significant on the bulk density of the extruded products ($p < 0.001$). While fish had a direct effect, extrusion temperature had an inverse effect on the bulk density of the extrudates. Effect of moisture was also significant ($p < 0.01$). Fish content accounted for 40% and extrusion

Table 3.5--ANOVA table for BKDEN

Mean squares	p	df	Term
118539	0.0025	1	Moisture
3.9512E+05	0.0000	1	Fish
774.8	0.7742	1	NFDM
1.6518E+05	0.0004	1	Barr_Temp
2049.8	0.6247	1	Moisture*Fish
19385.0	0.1454	1	Moisture*NFDM
68458.1	0.0113	1	Moisture*Barr_Temp
10222.2	0.2823	1	Fish*NFDM
81910.4	0.0065	1	Fish*Barr_Temp
2346.0	0.6010	1	NFDM*Barr_Temp
161.7	0.8938	1	Moisture^2
4832.4	0.4711	1	Fish^2
740.0	0.7752	1	NFDM^2
2081.6	0.6506	1	Barr_Temp^2
8217.67		15	Error

temperature accounted for 17% of the observed variability. Moisture content explained 11% of the variability in bulk density. Significant interaction effects were also found (Figure 3.1). At any extrusion temperature, the bulk density was affected by fish content. Increasing fish content provided an increased fish protein, which restricted the expansion of the product. Similarly, an increase in NFDM content increased dairy protein content and provided a compact structure, hence, a high bulk density. It was necessary to decrease the moisture content, fish content, and NFDM content in order to get an expanded snack.

Clayton and Miscourides (1992) reported an increase in bulk density of extrudates with an increase in fish content. Gautam *et al.* (1997) also reported a positive effect of fish solids on the bulk density of the extrudates containing fish.

Expansion ratio

The following model explained 86% of the variability due to the variation of the independent variables. Table 3.6 lists the mean squares for all the terms.

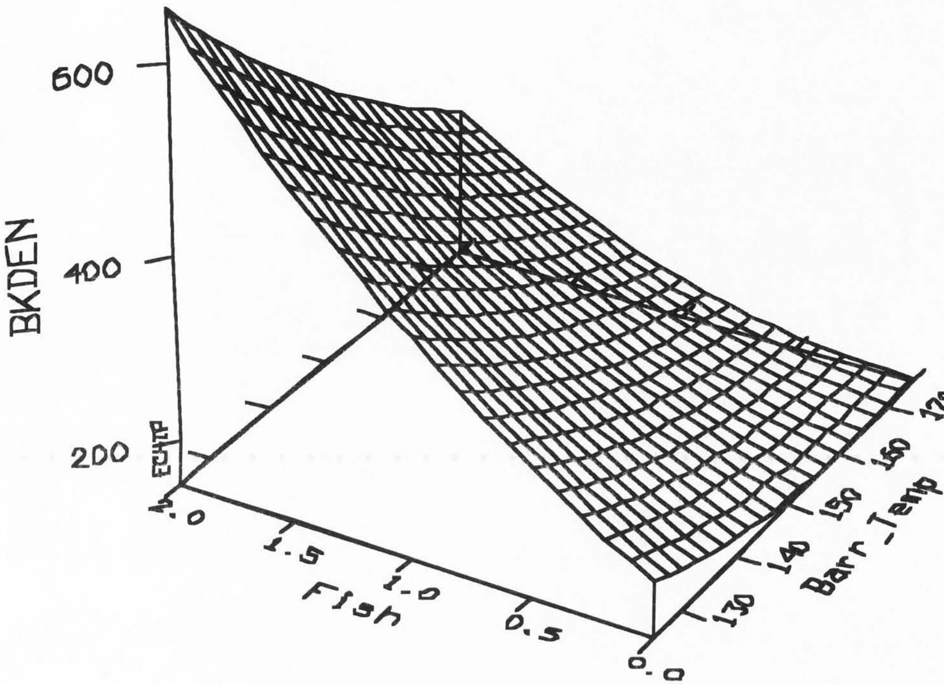

Figure 3.1--Effect of temperature and fish content on bulk density of the extrudates.

Table 3.6--ANOVA table for ER

Mean squares	p	df	Term
2.205	0.0005	1	Moisture
4.021	0.0000	1	Fish
0.012	0.7464	1	NFDM
0.011	0.7620	1	Barr_Temp
0.563	0.0414	1	Moisture*Fish
0.020	0.6831	1	Moisture*NFDM
0.490	0.0549	1	Moisture*Barr_Temp
1.082	0.0074	1	Fish*NFDM
1.323	0.0038	1	Fish*Barr_Temp
0.048	0.5229	1	NFDM*Barr_Temp
0.008	0.8143	1	Moisture^2
0.049	0.5299	1	Fish^2
0.002	0.8938	1	NFDM^2
0.061	0.4849	1	Barr_Temp^2
0.113		15	Error

$$ER = 3.66 - 0.07M - 0.47F + 0.038M*F + 0.104F*N + 0.115F*T \quad \text{...(5)}$$

Expansion ratio is an indication of the radial expansion of the product, and for snacks a higher expansion ratio is desired. The expansion process can be described as nucleation in the die, extrudate swelling immediately beyond the die, followed by bubble growth and collapse (Kokini *et al.,* 1991). Measured expansion ratios varied from a minimum of 1.98 (30% moisture, 2% Fish, 5% NFDM, and 125°C) to a maximum of 4.83 (20% moisture, 0% fish, 0% NFDM, and 125°C). The linear effect of moisture and fish was significant ($p < 0.01$). Fish and temperature had significant interaction effects (Figure 3.2) ($p < 0.01$). At any given extrusion temperature, the ER decreased as fish content increased. Fish and moisture also had a significant interaction effect ($p < 0.01$). Moisture and fish content had an inverse relation with the ER.

Decreased radial expansion was observed at increased moisture and protein levels. Similar reduction in expansion of the extrudate due to the increased fish content was observed by Gautam *et al.* (1997) and Clayton and Miscourides (1992).

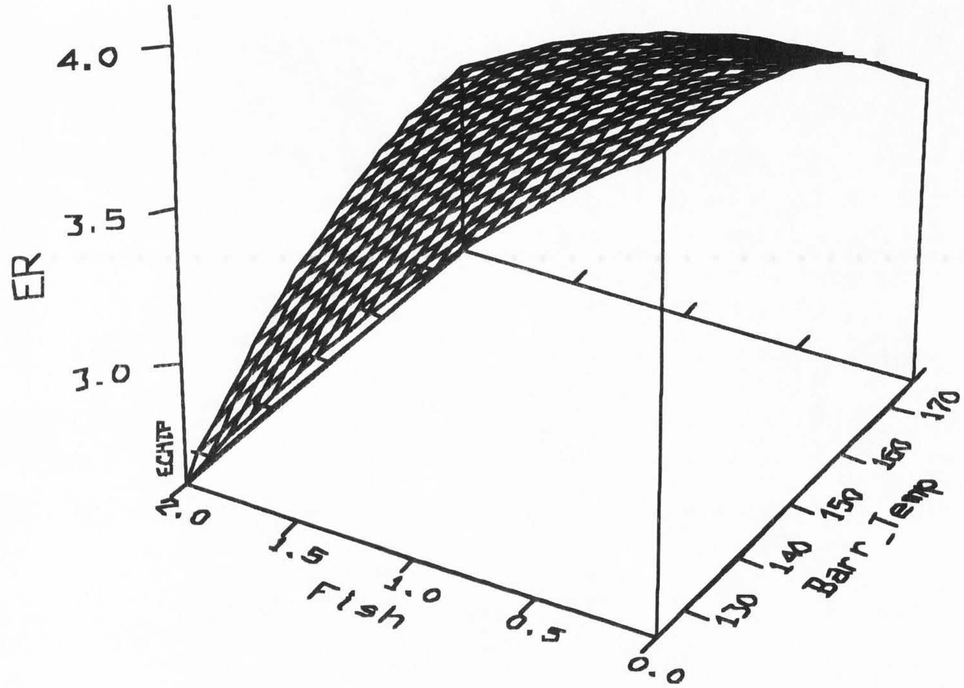

Figure 3.2--Effect of extrusion temperature and fish content on expansion ratio of the extrudates.

Shear stress

The following model explained 90% of the variability on the shear strength of the extrudates. Table 3.7 lists the mean squares for all the terms.

$$SS = 805.8 + 137.29\,M + 1184.9F - 235.9N - 34.16T - 40.79F*T \quad \ldots\ldots (6)$$

Shear stress is an important textural attribute of the expanded snack. A hard snack will have a high shear stress, and a soft snack will have a low shear stress. Measured shear stress values varied from a minimum value of 227.2 KPa (20% moisture, 0% fish, 5% NFDM, and 125°C) to a maximum value of 7479.9 KPa (30% moisture, 2% fish, 0% NFDM, and 125°C). Linear effects of temperature and fish were highly significant ($p < 0.001$). They explained about 42% of the observed variability. Moisture and NFDM content also had significant linear effects ($p < 0.01$). Lactose present in NFDM could have prevented amylose from forming a network resulting in a weak structure, thus decreasing SS. Interactive effects were found between fish and temperature (Figure 3.3).

Water absorption index

The following model explained 80% of the variations in WAI due to the variability of the independent

Table 3.7--ANOVA table for SS

Mean squares	p	df	Term
9.675E+06	0.0024	1	Moisture
2.516E+07	0.0000	1	Fish
6.383E+06	0.0078	1	NFDM
1.295E+07	0.0050	1	Barr_Temp
673200	0.3300	1	Moisture*Fish
673200	0.4799	1	Moisture*NFDM
2.602E+06	0.0664	1	Moisture*Barr_Temp
2.138E+06	0.0929	1	Fish*NFDM
1.664E+07	0.0002	1	Fish*Barr_Temp
560751	0.3727	1	NFDM*Barr_Temp
81411	0.7382	1	Moisture^2
1.845E+06	0.1266	1	Fish^2
2.688E+05	0.4558	1	NFDM^2
1.764E+05	0.9972	1	Barr_Temp^2
664005		15	Error

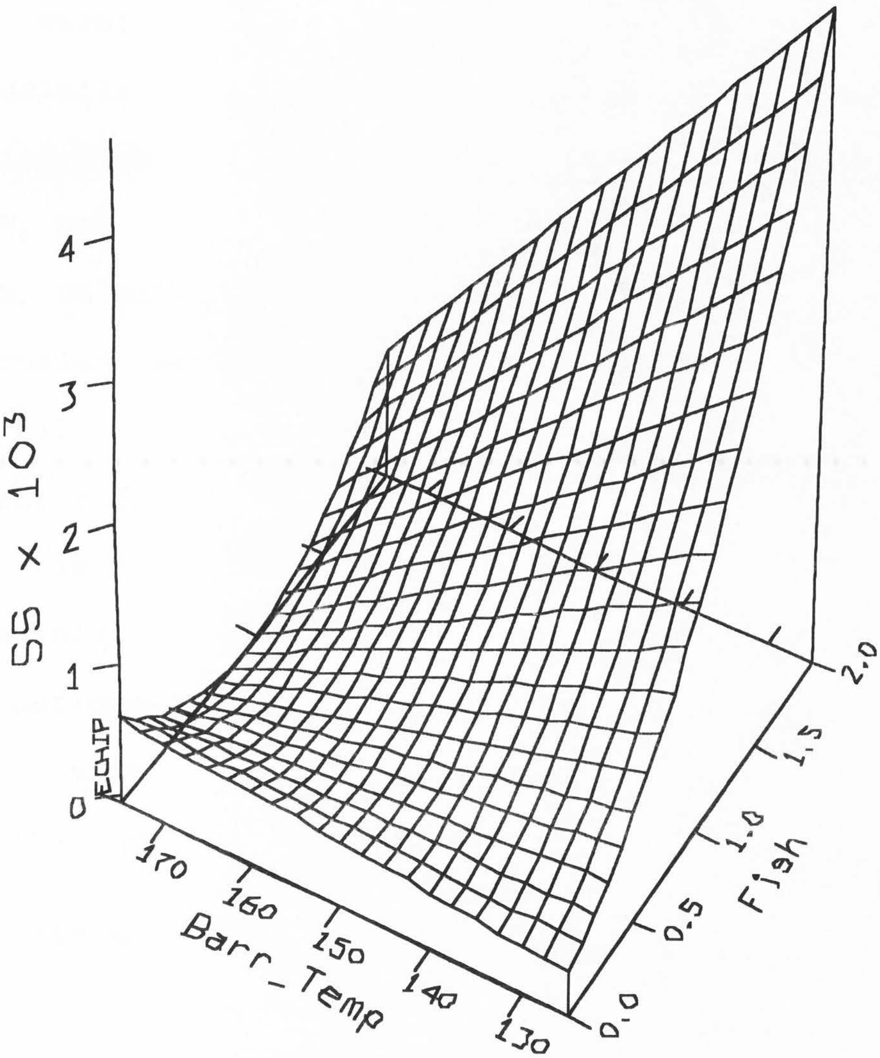

Figure 3.3--Effect of temperature and fish content on shear strength of the extrudates.

variables. Table 3.8 lists the mean squares for all the terms in the model.

$$WAI = 5.27 + 0.48M - 0.084N + 0.0093T \quad (7)$$

Water absorption index is an indicator of the degree of gelatinization of the extrudates. Measured WAI values varied from a minimum of 4.64 (20% moisture, 0% fish, 5% NFDM, and 125°C) to a maximum of 6.58 (30% moisture, 0% Fish, 0% NFDM, and 175°C). The linear effect of moisture, extrusion temperature and NFDM were significant on WAI ($p < 0.01$). While moisture and extrusion temperature had a direct effect, NFDM had an inverse effect on WAI.

Increased moisture increased the chances of gelatinization of starch, thus increasing WAI. Clayton and Miscourides (1992) also reported that temperature and moisture content were the most important variables in determining the WAI of fish extrudates.

Specific Mechanical Energy

The following model explained 94.3% of the variation in SME, due to the variation of independent variables. Table 3.9 lists the mean squares of all the terms.

$$SME = 0.194 - 0.004M - 0.02F + 0.003N - 0.0006T \quad (8)$$

Calculated values of SME varied from a minimum of 0.15 KW h/Kg (30% moisture, 2% fish, 0% NFDM, and 175°C)

Table 3.8--ANOVA table for WAI

Mean squares	p	df	Term
1.179	0.0021	1	Moisture
0.029	0.5691	1	Fish
0.810	0.0060	1	NFDM
1.058	0.0031	1	Barr_Temp
0.010	0.7254	1	Moisture*Fish
0.042	0.4744	1	Moisture*NFDM
2.50E-05	0.9860	1	Moisture*Barr_Temp
0.221	0.1132	1	Fish*NFDM
0.260	0.0879	1	Fish*Barr_Temp
0.021	0.6113	1	NFDM*Barr_Temp
0.201	0.1454	1	Moisture^2
0.022	0.5841	1	Fish^2
0.306	0.0618	1	NFDM^2
0.005	0.8157	1	Barr_Temp^2
0.078		15	Error

Table 3.9--ANOVA table for SME

Mean squares	p	df	Term
3.9813E-03	0.0000	1	Moisture
6.9975E-03	0.0001	1	Fish
8.7975E-04	0.0030	1	NFDM
3.6086E-03	0.0000	1	Barr_Temp
7.6563E-05	0.2921	1	Moisture*Fish
1.8906E-04	0.1067	1	Moisture*NFDM
1.5625E-06	0.8781	1	Moisture*Barr_Temp
1.5625E-06	0.8781	1	Fish*NFDM
7.6563E-05	0.2921	1	Fish*Barr_Temp
7.6562E-05	0.2921	1	NFDM*Barr_Temp
4.5310E-06	0.8689	1	Moisture^2
4.4210E-05	0.4561	1	Fish^2
4.1952E-05	0.4548	1	NFDM^2
4.1421E-05	0.4561	1	Barr_Temp^2
6.4203E-05		15	Error

to a maximum of 0.26 KW h/Kg (20% moisuture, 0% fish, 5% NFDM, and 125°C). All four independent variables had significant linear effects on the SME ($p < 0.01$). While fish content had a direct effect, moisture, temperature, and NFDM had an inverse effect on SME. Fish content explained 41%, and moisture content explained 23% of the total variability in SME. No significant quadratic or interaction effects were found between SME and the independent variables.

Specific mechanical energy (SME) is a function of temperature, moisture, and torque. As the moisture content increased, SME decreased. Increasing temperature could have increased the viscosity of the mix, resulting in lesser torque, which decreased SME. Gautam *et al.* (1997) also reported an inverse relationship between SME and fish content. They also attributed this effect to the lubricating effect of oil and protein in fish solids.

Product temperature

The following model, which neglects the insignificant terms, explained 99.5% of the total variation of product temperature. Table 3.10 lists the mean squares for each term.

Table 3.10--ANOVA table for PT

Mean squares	p	df	Term
60.072	0.0008	1	Moisture
25.836	0.0163	1	Fish
7.332	0.1828	1	NFDM
8996.010	0.0000	1	Barr_Temp
23.281	0.0192	1	Moisture*Fish
4.731	0.2554	1	Moisture*NFDM
0.226	0.7997	1	Moisture*Barr_Temp
0.601	0.6794	1	Fish*NFDM
0.141	0.8412	1	Fish*Barr_Temp
0.106	0.8621	1	NFDM*Barr_Temp
1.565	0.5102	1	Moisture^2
8.13E-05	0.9966	1	Fish^2
1.747	0.4899	1	NFDM^2
67.987	0.0003	1	Barr_Temp^2
3.382		15	Error

$$PT = 163.157 - 0.35M - 1.17F + 0.89T + 0.24\ M*F - 0.008T^2 \quad (9)$$

Observed product temperature varied from a maximum of 187°C (20% moisture, 0% Fish, 5% NFDM, and 175°C) to a minimum of 133.6°C (20% moisture, 2% fish, 0% NFDM, and 125°C). Linear effect of extrusion temperature and moisture was significant ($p < 0.001$). Extrusion temperature had a direct linear and quadratic effect, but moisture content had an inverse linear effect on product temperature. The negative effect of fish content was significant ($p < 0.05$). Increased moisture content reduced the friction between the barrel and the screws, thus reducing product temperature. Increased fish content provided an increased fat content, which also helped in the lubrication of the screws. Hence, both of these factors reduced the product temperature. Product temperature also had a significant interaction effect between moisture content and fish content ($p < 0.05$). Goga (1997) also reported a significant linear effect of temperature and moisture on the extrudates but in a corn-based flour system.

Die pressure

Neglecting insignificant terms, the following model explained 93.3% of variation in die pressure due to the variation of the independent variables. Table 3.11 lists the mean squares of the terms in the model.

$$DP = 3403.65 - 342.59M - 32.63T + 29.22\,M^2 \quad \text{.........(10)}$$

Measured values of die pressure varied from a minimum of 1034.21 KPa (30% moisture content, 0% fish, 5% NFDM, and 175°C) to a maximum of 6825.80 KPa (20% moisture, 0% fish, 5% NFDM, and 175°C). The linear effect of temperature and moisture on the die pressure was significant ($p < 0.001$). Moisture accounted for 68%, and temperature accounted for 14% of the variability in the model. Moisture also had a significant quadratic effect on the die pressure ($p < 0.05$). Addition of fish and NFDM did not have any significant effect on die pressure.

Both linear terms of temperature and moisture had negative effects on die pressure. As the moisture content increased, the viscosity of the mix probably decreased, making it easy to pass through the die orifice. Similarly, the increased temperature probably increased the melt, thereby decreasing the viscosity and the die pressure of the extrudates. Goga (1997) also reported a negative effect of moisture and temperature on die pressure in a corn-based extrusion system.

Table 3.11--ANOVA table for DP

Mean squares	p	df	Term
5.756E+07	0.0000	1	Moisture
1632941	0.0556	1	Fish
368125	0.3665	1	NFDM
1.20E+07	0.0000	1	Barr_Temp
451786	0.2886	1	Moisture*Fish
35915.9	0.7607	1	Moisture*NFDM
601517	0.2236	1	Moisture*Barr_Temp
285610	0.3955	1	Fish*NFDM
1.671E+06	0.0515	1	Fish*Barr_Temp
451786	0.2886	1	NFDM*Barr_Temp
1803424.9	0.0456	1	Moisture^2
363151	0.3700	1	Fish^2
294081.1	0.4186	1	NFDM^2
539815.28	0.2485	1	Barr_Temp^2
373213		15	Error

CONCLUSIONS

Moisture content had a highly significant linear effect on all measured attributes of the extrudates, which were expansion ratio (ER), bulk density (BKDEN), specific mechanical energy (SME), product temperature (PT), die pressure (DP), shear strength (SS), and water absorption index (WAI) ($p < 0.001$). Extrusion temperature was found to have a highly significant effect ($p < 0.001$) on all measured attributes except ER. Fish content had a highly significant effect on the physicochemical attributes SS, BULKDEN, ER, and SME. NFDM was found to have a significant effect on SS, WAI, and SME.

In general, as the fish and moisture content increased, bulk density and shear stress of the extrudates increased, making them unacceptable for a snack food. As the extrusion temperature increased, well expanded lighter extrudates were obtained. From the experiments that contained fish, the following conditions gave a uniformly expanded extrudate of low bulk density: moisture 20%, NFDM 0%, fish 2%, and processing temperature 175°C.

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CHAPTER IV

UTILIZATION OF RAINBOW TROUT FRAMES IN FISH CRACKERS (KEROPOK)[1]

Summary

Preliminary studies were conducted using deboned rainbow trout (*Oncorhynchus mykiss*) to make fish crackers. Fish mince obtained from trout frames was mixed with either wheat flour, tapioca flour, or tapioca starch to make the cracker. Crackers made with wheat flour were not acceptable based on our subjective evaluation. Panelists evaluated crackers containing tapioca flour and starch for texture and taste. The mean sensory rating for texture was 6.38 and 6.07 and for taste was 5.81 and 5.61 for crackers made with tapioca starch and tapioca flour, respectively. There was no difference in texture and taste scores between tapioca flour and tapioca starch. Taste panel studies indicate a good potential for the product.

Introduction

Fish has long served as a source for animal protein for people living close to the sea. Fish protein is

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[1]Coauthored by S. Muralidharan and C. Hansen.

balanced in essential amino acid composition and is easily digested (Karmas & Lauber, 1987). Fish contains relatively high amounts of polyunsaturated fatty acids, and fish is relatively low in calories and cholesterol. Consumers' growing preference for nutritional and healthy food is creating a large demand for processed fish food in the U.S.A.

Rainbow trout (*Oncorhynchus mykiss*) is a fresh water fish raised commercially in Utah and Idaho. These two states account for over 80% of the total trout production in the U.S.A. During 1996, the annual production of trout amounted to 53.6 million pounds valued at $56.9 million (U.S. Bureau of the Census, 1997). Much of the trout produced in the USA is processed into fillets. With the current processing technology, about one-half of a filleted fish is discarded as waste in the form of head, tail, and frames. Flesh in frames is about 25% of total waste, and it can be harvested by deboning. The deboned meat can be processed into snack foods, thereby decreasing the volume of the waste to be treated. However, very little information is available in the literature for the utilization of deboned trout mince.

Venugopal and Shahidi (1995) reviewed the possibilities of making value-added products using underutilized fish species as a starting material. Deboned fish can be processed into various foods such as fish sticks, fish fingers, fish cakes, fish balls, and snack products such as crackers (Karmas & Lauber, 1987; Maga & Reddy, 1985; Meinke *et al.,* 1983; Reddy *et al.*, 1990). Many Malaysian researchers have made a cracker called keropok using native fishes (Yu *et al.,* 1981; Siaw *et al.*, 1985; Yu, 1993). Keropok is produced by mixing shrimp or fish flesh with starch and water to form a dough that is shaped, cooked, and then sliced. The slices are then dried and expanded into a low-density porous product upon heating in hot oil (Siaw *et al.*, 1985; Yu, 1993). Keropok is similar to potato chips and represents a good market potential. However, no such product containing fish mince is readily available in the local market.

The objective of this study was to explore the suitability of deboned trout flesh in the development of a fish cracker.

Materials and Methods

Materials

Rainbow trout (*Oncorhynchus mykiss*) frames were obtained from Paradise Farms, Paradise, Utah, and were deboned using a twin screw mechanical deboner (model RSTC-02 BX-VO5, Beehive Machinery Inc., Sandy, Utah). Deboned fish flesh was collected in polyethylene bags of 2 Kg size and stored at -28.8°C (-20°F) until further use. Tapioca starch (Combine Thai Foods Co. Ltd., Bangkok), Tapioca flour(Now Foods Inc., Glendale Heights, Illinoise), wheat flour (Pillsbury Inc., Minneapolis, Minnesota), salt, and spices were obtained from the local market.

Methods

Proximate composition of the deboned fish was determined using the standard AOAC(1990) procedures. The ether extraction method as described by procedure 960.39B was used for the determination of fat content. Vacuum oven method as described by procedure 950.46A was used to determine moisture content. Oxidation method using a muffle furnace was used to determine the ash content (procedure 920.153). Because fish does not contain any carbohydrates, the protein content was determined as the difference between 100 - (percentage composition moisture, fat and ash).

Frozen deboned fish mince was thawed for 24 h at 4°C before being processed. Procedure for making keropok described by Siaw *et al.* (1985) was followed with modifications for this process. The procedure is summarized in Fig. 4.1. A ratio of 30:70 (Yu *et al.*, 1981;

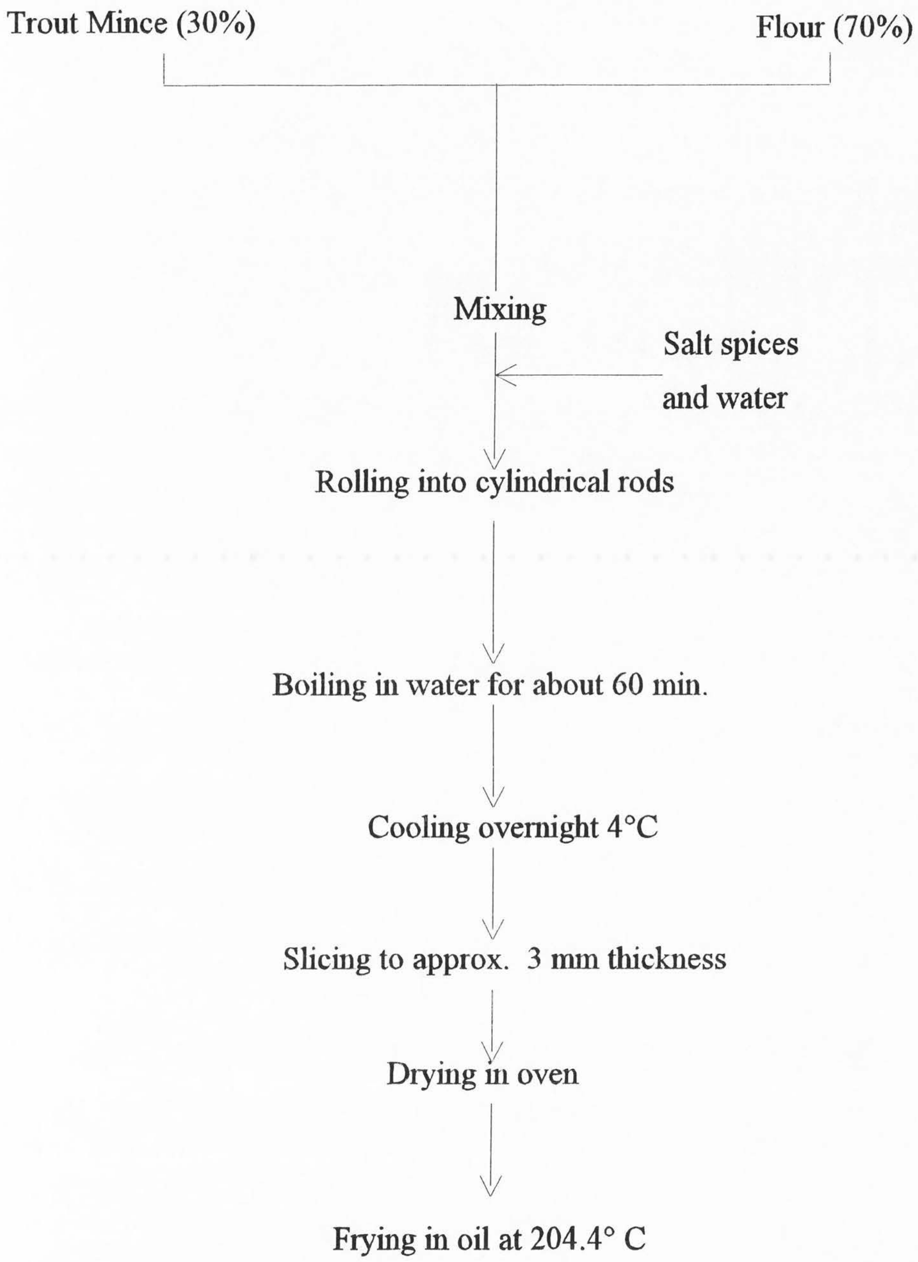


Figure 4.1 Fish cracker processing of minced trout.

Choudhury & Gogoi, 1995) fish mince to the flour was selected for this study. The mixture was thoroughly blended using a blender (Sunbeam Corp., Delray Beach, Florida). About 30% water was added to get the required dough consistency. Salt and spices were added to mask the fishy flavor and give a good taste. The composition of the spices is proprietary.

The dough was manually shaped into cylindrical rods of about 2 cm diameter and 15 cm length. More flour was added to the dough at this stage to prevent it from sticking to the board. Shaped rods were immersed in boiling water for an hour to fully gelatinize the starch/fish mixture. Cooked rods were cooled and chilled at 4°C overnight and sliced to 3-mm (Siaw *et al.*, 1985, Yu, 1993) thick pieces using a slicer (model #919, Berkel Inc., LaPorte, Indiana). Slices were dried in an oven (model#1640 Shelldon Manufacturing Inc., Cornelius, Oregon) for 8-10 h at 60°C (140°F) and to a final moisture content of less than 10% on w.b. Dried slices were cooked in vegetable oil held at a temperature of 204.4°C (400°F).

The crackers were analyzed for the following physicochemical parameters.

Linear expansion

Percentage linear expansion was calculated using the formula

$$\text{linear expansion (\%)} = \frac{D_2 - D_1}{D_1} \times 100$$

where

D_2: Diameter of the product after cooking

D_1: Diameter of the product before cooking

as reported by Yu (1993). The reported result is the average of 10 samples.

Bulk density

Bulk density of the cracker was determined using the method reported by Lue *et al.* (1991). Approximately 10 g of the whole crackers was placed in a beaker of known weight and volume. White sand of known density was poured into the remaining space in three to four increments. The beaker was tapped after each increment to ensure the packing of sand in the remaining space and was leveled off using a straight edge. Based on the difference of weight of sand with and without the crackers, the volume of cracker was calculated. This volume was used in the determination of bulk density of the crackers. The reported result is the average of five samples.

Water absorption index

Water absorption index (WAI), which is also a measure of the degree of gelatinization of the product, was determined using the procedure reported by Anderson *et al.* (1969). A 2.5-g sample of the product was ground (< 60 mesh) and suspended in 30 ml of water at 30°C in a 50-ml tared centrifuge tube. The product was kept in a water bath for 30 min and stirred intermittently. After 30 min it was centrifuged at 3000 g for 10 min and the supernatant poured off. The weight of gel per 1 g of dry sample was the WAI. The reported result is the average of four samples.

Composition

Proximate composition of the crackers was determined using the standard AOAC procedures (1990). Vacuum oven method was used to determine moisture content. Modified babcock method was used to determine the fat content. Protein content was determined using the Kjeldahl method. Ash content was determined using the muffle furnace. The results are the average of three replicate samples.

Sensory panel studies

Sensory testing of the crackers made using tapioca starch and tapioca flour was conducted at the Nutrition and Food Sciences Department of Utah State University. Panelists rated the product's taste and texture on a scale of 1 to 9, where a score of 9 = like the product very much and 1 = disliked very much. Panelists were also asked to comment on the texture and taste of the fish crackers. One hundred fourteen untrained panelists participated. Scores were compared using Student's t test.

Results and Discussion

The proximate composition of the deboned fish frames is shown in Table 4.1.

Our subjective evaluation of the products during the preliminary studies showed that wheat flour was not suitable for the fish cracker. The product had the least expansion and a hard texture. This could be due to the high protein content of the wheat flour. Similar results were observed by Yu (1991) and Julianty *et al.* (1994). Crackers made using either tapioca flour or tapioca starch had a good expansion characteristic and crunchy texture. Hence, the taste panel studies were limited to the crackers made from tapioca flour and tapioca starch.

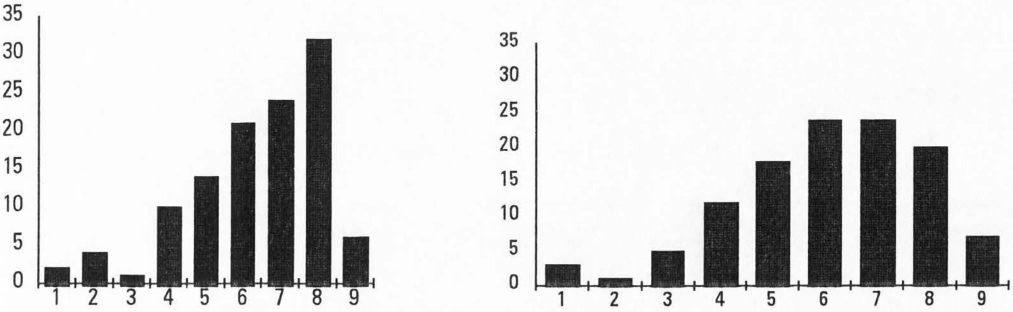
Figures 4.2 and 4.3 are the histograms obtained for the taste and texture of the fish crackers made using tapioca starch and tapioca flour. Average texture score

Table 4.1 Proximate composition of trout mince

Composition	Percentage (Mean±SD)
Moisture	72.7±0.3
Fat	10±2
Protein	17±1
Ash	0.73±0.01

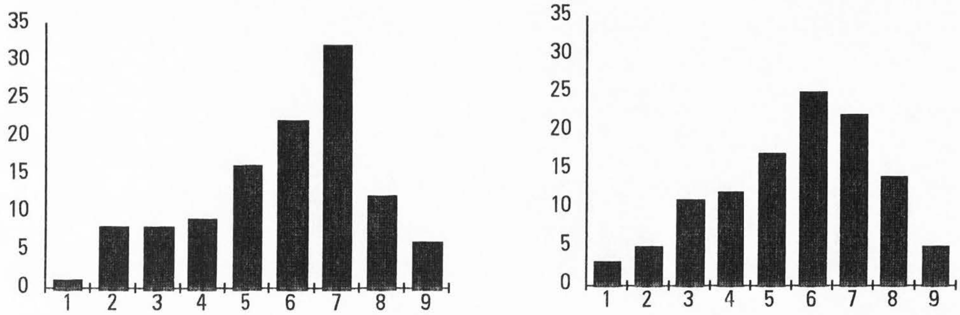
for the cracker made of tapioca starch was 6.38 and that of tapioca flour was 6.07. Average taste scores for the crackers were 5.81 and 5.61, respectively. Both the products were liked by a majority of the the panelists, and there was no significant difference in taste or texture scores for the crackers at the 95% confidence level. Most of the 114 panelists rated 7 or above for the texture of the cracker made using tapioca starch.

Comments of the panelists are an important indication about the marketability of the product. Some commented that the product texture was very crispy or crispy. Many noted that the product was similar to corn chips and potato chips, and said the product did not really taste like fish. This is important as many American consumers do not like a strong, fishy flavor. Based on the cost factor, additional studies are being conducted using



(a) (b)

Figure 4.2 Comparison of texture scores of keropok made using (a) tapioca starch and (b) tapioca flour.



(a) (b)

Figure 4.3 Comparison of taste scores for keropok made using (a) tapioca starch and (b) tapioca flour.

tapioca starch in the commercial production of the cracker.

Physicochemical properties

Fish crackers made using the tapioca starch had the following physicochemical properties (mean±SD).

Percentage linear expansion	= 69 ± 9%
Water absorption Index	= 5.22 ± 0.08
Bulk Density	=0.26 ± 0.02 gm/cc
Fat Content	=28 ± 1 %
Protein Content	=6 ± 1%
Ash Content	=1.60 ± 0.07%
Moisture content	=3.53 ± 0.12%

Conclusion

Trout meat obtained by mechanically deboning fish frames of rainbow trout has been used in the development of a fish cracker similar to keropok. Local fish processors have indicated an interest in producing this kind of product. Further study is under way to develop a procedure to manufacture the product on a large scale.

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CHAPTER V

SUMMARY

Most of the fish industry's catch of unconventional species is wasted, due to lack of available technology to utilize them. Recent research has concentrated on converting mince obtained from those species into value-added products. The states of Utah and Idaho account for more than 80% of the production of rainbow trout (Oncorhynches mykiss). Currently the wastes from the filleting operation of this fish are dumped onto landfills, adding to environmental pollution. Because of the recent increase in demand for low fat, high protein snack foods, this research explored the possibility of utilizing fish mince in an expanded snack food. Twin screw extrusion technology and conventional technology were evaluated.

In the extrusion technology, response surface methodology was employed in the design and evaluation of studies looking at the effect of moisture, fish, NFDM content, and process temperature on the physicochemical properties of the extruded snack. Response surfaces were generated to visualize the effects of the above-mentioned variables. From the extrusion runs, favorable conditions of the process that could lead to the development of an expanded fish snack food were identified (moisture:20% [w.b]), fish:2%, NFDM:0%, and temperature:175°C). Further research in the form of flavoring of the snack could be conducted to improve the acceptance of the product.

Conventional technology was used to develop a cracker called keropok containing trout mince. The effects of different types of starches (wheat flour, tapioca flour, and tapioca starch) on the acceptability of the snack were studied. Tapioca starch gave a well expanded snack. Taste panel studies conducted on the developed snack revealed good potential for the snack. Some local fish processors have expressed interest in the development of such a product, based on the taste and texture of the experimentally developed cracker. Further research may be conducted on the development of a continuous process to manufacture the cracker on a large scale.

APPENDICES

Appendix A: Tables

Table A.1. Experimental Data for Responses

Trials	P.T (°C)	DP (Kpa)	SME (KW h/Kg)	WAI	SS (Kpa)	BKDEN (Kg/m^3)	ER
1	182.2	4205.79	0.22	5.92	244.13	81.51	4.75
2	165.3	3102.63	0.21	5.51	950.80	236.86	3.87
3	137.1	6550.00	0.22	5.66	1286.31	340.57	3.56
4	187.3	3309.48	0.23	5.88	356.30	133.70	3.36
5	138.5	6825.80	0.25	5.52	562.75	150.87	4.83
6	179.6	6618.96	0.17	5.72	2015.44	451.85	2.74
7	178.6	5791.59	0.20	5.26	722.44	319.61	3.25
8	167.4	6343.17	0.21	5.10	804.30	233.46	3.85
9	164.4	3309.48	0.20	4.89	496.76	239.94	3.89
10	134.5	3171.24	0.22	5.34	762.38	337.02	3.37
11	161.8	3240.53	0.19	5.67	704.38	300.50	3.48
12	178.3	1516.85	0.18	6.58	1905.33	232.52	3.28
13	162.1	3516.33	0.17	6.05	2109.07	344.35	3.41
14	179.5	1378.95	0.15	5.96	709.10	272.09	3.55
15	178.5	1034.21	0.18	5.70	1562.74	274.89	2.76
16	139.2	5929.49	0.26	4.64	227.29	174.80	4.60
17	135.0	2826.85	0.20	5.94	1740.82	251.09	3.81
18	165.1	2826.85	0.20	5.18	429.91	227.60	3.94
19	164.0	3378.43	0.20	5.20	685.62	273.79	3.58
20	160.9	3309.48	0.17	5.31	2663.09	422.43	3.08
21	163.7	6067.38	0.21	5.32	551.23	203.59	4.02
22	157.4	1861.58	0.17	5.86	997.29	315.16	3.30
23	134.8	5171.06	0.21	4.76	1187.94	415.03	3.12
24	134.1	3309.48	0.17	5.83	6960.72	1008.33	1.99
25	164.7	2964.74	0.21	5.04	723.85	199.21	3.95
26	180.4	2688.95	0.17	5.70	585.40	175.91	3.88
27	133.6	5171.06	0.19	5.33	5419.89	560.70	2.67
28	134.5	3654.22	0.17	5.85	5479.94	798.98	2.27
29	162.4	3516.32	0.19	5.10	695.66	279.53	3.57
30	179.2	1378.95	0.15	6.33	2157.05	329.07	3.09

Table A.2. Summary of Results for the extruded fish snack

PT	DP	SME	WAI	SS	BK DEN	ER	Parameter
***	***	***	**	**	**	***	1 Moisture
*	.	***	.	***	***	***	2 Fish
.	.	**	**	**	.	.	3 NFDM
***	***	***	**	***	***	.	4 Barr_Temp
*	.	.	.	.	.	*	5 Moisture*Fish
.	.	.	.	.	.	.	6 Moisture*NFDM
.	.	.	.	.	*	.	7 Moisture*Barr_Temp
.	.	.	.	.	.	**	8 Fish*NFDM
.	.	.	.	***	**	**	9 Fish*Barr_Temp
.	.	.	.	.	.	.	10 NFDM*Barr_Temp
.	*	.	.	.	.	.	11 Moisture^2
.	.	.	.	.	.	.	12 Fish^2
.	.	.	.	.	.	.	13 NFDM^2
***	.	.	.	.	.	.	14 Barr_Temp^2

Where

*** : $p < 0.001$

** : $p < 0.01$

* : $p < 0.05$

. : $p > 0.05$

Appendix B: Figures

```
 _____________________________________________________
| .   .   .   .   .   .   .   .   .   .   .   .  |-*-||   4  Barr_Temp
| .|-o-|.   .   .   .   .   .   .   .   .   .   .   .|  14  Barr_Temp^2
| |-o-| .   .   .   .   .   .   .   .   .   .   .   .|   1  Moisture
||-*-|  .   .   .   .   .   .   .   .   .   .   .   .|   5  Moisture*Fish
||-o-|  .   .   .   .   .   .   .   .   .   .   .   .|   2  Fish
|-*-|.  .   .   .   .   .   .   .   .   .   .   .   .|   3  NFDM
x                                                    |   6  Moisture*NFDM
x                                                    |  13  NFDM^2
x                                                    |  11  Moisture^2
x                                                    |   8  Fish*NFDM
x                                                    |   7  Moisture*Barr_Temp
x                                                    |   9  Fish*Barr_Temp
x                                                    |  10  NFDM*Barr_Temp
x                                                    |  12  Fish^2
|____________________________________________________|
 |                         |                         |
 0                      23.274                    46.548
```

Figure B.1. Effects graph for product temperature.

```
 _________________________________________
| .  .  .  .  .  .  .  .  .  |-----o-----||  1  Moisture
| .  .  .  |-----o-----|  .  .  .  .  .  .|  4  Barr_Temp
||------*------| .  .  .  .  .  .  .  .  .| 11  Moisture^2
|------*------|  .  .  .  .  .  .  .  .  .|  9  Fish*Barr_Temp
|-----*------|.  .  .  .  .  .  .  .  .  .|  2  Fish
|----*-------|.  .  .  .  .  .  .  .  .  .| 14  Barr_Temp^2
xxxx                                      |  7  Moisture*Barr_Temp
xxxx                                      | 12  Fish^2
xxxx                                      | 10  NFDM*Barr_Temp
xxxx                                      |  5  Moisture*Fish
xxxx                                      | 13  NFDM^2
xxx                                       |  3  NFDM
xxx                                       |  8  Fish*NFDM
x                                         |  6  Moisture*NFDM
|_________________________________________|
 |                   |                   |
 0               2014.103             4028.207
```

Figure B.2. Effects graph for die pressure.

```
 _________________________________________________
| . . . . . . . . .|------o------||   1  Moisture
| . . . . . . . .  |------o-----| .|  2  Fish
| . . . . . |------o------|. . . . .| 4  Barr_Temp
| . |------*------| . . . . . . . .|  3  NFDM
|-----o------|. . . . . . . . . . .|  6  Moisture*NFDM
xxxx                               |  5  Moisture*Fish
xxxx                               |  9  Fish*Barr_Temp
xxxx                               | 10  NFDM*Barr_Temp
xxxx                               | 13  NFDM^2
xxxx                               | 12  Fish^2
xxxx                               | 14  Barr_Temp^2
x                                  | 11  Moisture^2
x                                  |  8  Fish*NFDM
x                                  |  7  Moisture*Barr_Temp
|__________________________________|
 |                 |                 |
 0               0.0244            0.0489
```

Figure B.3. Effects graph for specific mechanical energy.

```
 _____________________________________________
| .  .  .  |-------------*-------------||   1  Moisture
| .  .  . |-------------*-------------|.|   4  Barr_Temp
| .  .  |-------------o-------------|  .|   3  NFDM
|-----------------*-----------------|  .|  13  NFDM^2
|-------------o--------------| .  .  .  .|   9  Fish*Barr_Temp
|------------*---------------| .  .  .  .|  11  Moisture^2
|------------*--------------|  .  .  .  .|   8  Fish*NFDM
xxxxxx                                   |   6  Moisture*NFDM
xxxxxx                                   |  12  Fish^2
xxxxx                                    |   2  Fish
xxxx                                     |  10  NFDM*Barr_Temp
xxx                                      |   5  Moisture*Fish
xxx                                      |  14  Barr_Temp^2
x                                        |   7  Moisture*Barr_Temp
|________________________________________|
 |                  |                   |
 0                0.377               0.754
```

Figure B.4. Effects graph for water absorption index.

```
 _________________________________________
| .  .  .  .  .  .  |----------*----------||    2  Fish
| .  .  .  .  .  .|----------o----------|. .|   9  Fish*Barr_Temp
| .  .  .  .  .|---------o---------|  .  .  .|  4  Barr_Temp
| .  .  |---------*---------|.  .  .  .  .  .|  1  Moisture
| . |----------o----------|  .  .  .  .  .  .|  3  NFDM
|----------o----------|.  .  .  .  .  .  .  .|  7  Moisture*Barr_Temp
|----------*-----------| .  .  .  .  .  .  .|  12  Fish^2
|---------o----------| .  .  .  .  .  .  .  .|  8  Fish*NFDM
xxxxxx                                       |   5  Moisture*Fish
xxxxx                                        |  13  NFDM^2
xxxxx                                        |  10  NFDM*Barr_Temp
xxxx                                         |   6  Moisture*NFDM
xx                                           |  11  Moisture^2
x                                            |  14  Barr_Temp^2
|____________________________________________|
 |                   |                   |
 0               1594.289            3188.578
```

Figure B.5. Effects graph for shear strength.

```
 _______________________________________________
| .  .  .  .  .  .  . |---------*---------||   2  Fish
| .  .  .  |---------o---------|.  .  .  .|   4  Barr_Temp
| .  . |--------*--------|.  .  .  .  .  .|   1  Moisture
| .  |---------o---------|.  .  .  .  .  .|   9  Fish*Barr_Temp
| . |---------o---------| .  .  .  .  .  .|   7  Moisture*Barr_Temp
|-------*---------| .  .  .  .  .  .  .  .|   6  Moisture*NFDM
xxxxxx                                    |   8  Fish*NFDM
xxxxx                                     |  12  Fish^2
xxx                                       |  14  Barr_Temp^2
xxx                                       |  10  NFDM*Barr_Temp
xxx                                       |   5  Moisture*Fish
xx                                        |  13  NFDM^2
xx                                        |   3  NFDM
x                                         |  11  Moisture^2
|_________________________________________|
 |                   |                   |
 0                193.765             387.531
```

Figure B.6. Effects graph for bulk density.

```
 ______________________________________________________________
|  .    .    .    .    .    .  |----------o----------||   2  Fish
|  .    .    .    .|---------o---------|    .    .    .|   1  Moisture
|  .    .|-----------*-----------|  .    .    .    .|   9  Fish*Barr_Temp
|  .    .|----------*----------|.    .    .    .    .|   8  Fish*NFDM
||----------*----------|    .    .    .    .    .    .|   5  Moisture*Fish
|----------*-----------|    .    .    .    .    .    .|   7  Moisture*Barr_Temp
xxxxx                                                  |  14  Barr_Temp^2
xxxxx                                                  |  12  Fish^2
xxxx                                                   |  10  NFDM*Barr_Temp
xxx                                                    |   6  Moisture*NFDM
xx                                                     |   3  NFDM
xx                                                     |   4  Barr_Temp
xx                                                     |  11  Moisture^2
x                                                      |  13  NFDM^2
|______________________________________________________|
 |                        |                         |
 0                      0.642                     1.283
```

Figure B.7. Effects graph for expansion ratio.